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Clinical characteristics and mutation profiles of Collagen VI–related myopathy

Collagen VI 연관 근병증의 임상 양상 및 유전자 변이에 대한 연구

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ABSTRACT

Background: collagen VI-related myopathy is one of the most common type of congenital muscular dystrophy. This study was aimed to describe clinical, genetic characteristics, and identify their association of collagen VI-related myopathy patients.

Methods: We enrolled 23 patients with collagen VI-related myopathy who were confirmed to have pathogenic mutation of *COL6A1, COL6A2, COL6A3*. Medical record was reviewed retrospectively.

Results: Among 23 cases, 10 patients (43%) had congenital orthopedic problems such as torticollis, congenital hip dislocation or arthrogryposis congenita. Three patients (13%) never walked and 7 (35%) lost their walking ability later at the mean age of 10. Hyperlaxity of distal joints, contracture of proximal joints, and scoliosis were noted each in 12 (52%) patients. Six patients (26%) used mechanical ventilator support. Clinical features and courses varied by patients. Patients were categorized as Ullrich

type in 11 (48%), Bethlem type in 4 (17%), limb-girdle type in 3 (13%), and undetermined in 5 (22%). All patients showed sarcolemma-specific collagen VI deficiency in immunohistochemistry of muscle. Mutations for *COL6A1*, *COL6A2*, and *COL6A3* were found each in 15 (65%), 3 (13%), and 5 (22%) of each group of patients. All had heterozygous variants and most of mutations located in triple helical domain. Five novel variants were detected. There was no clear association between genotype and phenotype. Multiple congenital orthopedic problems including arthrogryposis multiplex congenital were frequently noted in early-severe group.

Conclusion: We verified heterogeneity in clinical features of collagen VI myopathy as well as in pathology and genotype. Multiple congenital orthopedic problems might suggest poor prognosis, without clear genotpye or phenotype association to clinical severity.

Keywords: Collagen VI related myopathy, Ullrich congenital muscular dystrophy, Bethlem myopathy, *COL6A1, COL6A2,*

COL6A

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LIST OF ABBREVIATIONS

CMD, congenital muscular dystrophy

COL6 myopathy, collagen VI-related myopathy

UCMD, Ullrich type congenital muscular dystrophy

BM, Bethlem myopathy

LGMD, limb-girdle muscular dystrophy

SSCD, sarcolemma-specific collagen VI deficiency

THD, triple helical domain

IHC, immunohistochemistry

CD, complete deficiency

INTRODUCTION

Congenital muscular dystrophies (CMDs) are genetically and phenotypically heterogeneous group of disorders characterized by early onset weakness or motor development delay with dystrophic change on muscle biopsy. Its heterogeneity have made detailed diagnosis very challenging, yet the diagnosis is crucial for patients' management, predicting the prognosis and counseling the patient and family due to properties such as lifelong disease course without curable treatment. Traditionally, CMDs had been classified according to pathologic and clinical features. However, development in the knowledge of genetic backgrounds has allowed a novel classification of CMDs according to genetic defect [1]. Among them, collagen VI-related myopathy (COL6 myopathy) is a genetically classified subtype of CMDs caused by mutation of COL6 genes, *COL6A1*, *COL6A2*, *COL6A3*. Each genes encode for three α-chains of collagen VI, and each chains are assembled into collagen VI protein. Collagen VI is an extracellular matrix protein found in most of tissues, and its absence or aberrant formation in muscle cause myopathies. Most well-known phenotype of COL6

myopathy is Ullrich congenital muscular dystrophy (UCMD, MIM 254090), characterized by early onset muscle weakness, hyperlaxity of the distal joint, contracture of proximal joint, scoliosis and respiratory insufficiency [2]. Bethlem myopathy (BM, MIM 158810) is the other form of COL6 myopathies [3-5], considered as milder phenotype, mainly characterized by joint contractures (commonly finger flexors, wrist, elbow and ankle) with slowly progressive muscle weakness mainly starting in the first 2 decades of life [4, 6]. It is well known that there are no clear clinical criteria to distinguish two phenotypes, and each phenotype is on each end of clinical continuum. Moreover, limb-girdle muscular dystrophy (LGMD) or myosclerosis myopathy were reported as other phenotypes of COL6 myopathy [7, 8]. The clinical boundaries between phenotypes have become more blurred according to expanding and overlapping spectrum, on the strength of recently growing genetic testing and increased number of reports. In genetic backgrounds, the mutation on same domain caused different phenotype, and both autosomal dominant and recessive patterns were observed in either UCMD or BM [9-12]. There have been many studies for each UCMD or BM, though clinical reports on overall clinical and genetic aspects of

COL6 myopathies were still limited [13]. We have therefore attempted to descript the various clinical spectrum of COL6 myopathies as well as genetic backgrounds, and to determine the predictors for outcome.

PATIENTS AND METHODS

1. Patients and phenotype classification

From August 2000 to July 2016, we screened patients with CMD unclassified by conventional diagnostic tools in Seoul National University Children's Hospital. Total 156 patients had targeted gene panel sequencing with or without Sanger confirmation of their parents. Among them, 23 patients who were confirmed to have pathogenic mutation in *COL6A1*, *COL6A2* or *COL6A3* were enrolled. The medical records including past medical history, physical examination findings, laboratory test, radiographic findings, and functional studies such as echocardiography or pulmonary function test and muscle pathology were reviewed. Informed consent for genetic testing was obtained in all patients and the study was approved by the Institutional Review Board (IRB) of the Seoul National University Hospital. (IRB No. 1610-080-799)

We classified the patients into 4 phenotypes, UCMD, BM, LGMD and undetermined, for identifying clinical spectrum of disease. The Patients with UCMD were defined as congenital or infantile onset progressive muscle weakness, hyperlaxity of distal joint,

contracture of proximal joint with relatively early-onset of scoliosis and loss of their walking ability. Subgrouping of BM was based on the clinical features including muscle weakness predominantly in the proximal and axial muscle, early joint contractures especially in finger flexor, wrist, or ankle and relative preserved motor performance without ventilator support. We categorized LGMD phenotype apart from BM, in the patients with proximal, axial, especially neck muscle weakness without joint contractures or rigid spine. The patients who did not belong in any three groups were classified into undetermined group.

Congenital hip dislocation was defined as unilateral or bilateral dislocation of hip joint, which was thought to be occurred their birth, arthrogryposis multiplex congenita and congenital torticollis were diagnosed by pediatric orthopedic doctors after patient's birth. All neurological assessment was performed by a qualified pediatric neurologist. Immunohistochemistry for collage VI was performed in 20 patients and reviewed by the same pediatric neurologist.

2. Targeted genes analysis

Sequencing analysis was performed in separate 2 centers, Seattle Children's Hospital and Seoul National University

Children's Hospital. Eleven patients (patient No.1, 7, 8, 9, 10, 11, 12, 13, 15, 16, and 18) have sequencing for target genes at Seattle Children's Hospital and remained patients (patient No. 2, 3, 4, 5, 6, 14, 17, 19, 20, 21, 22, and 23) in Seoul National University Hospital. Sequencing data from Seattle Children's Hospital was included in the previous study [13]. The target genes for various neuromuscular diseases were included in both centers; CMDs, congenital myopathies, metabolic and mitochondrial myopathies, storage myopathies, distal myopathies, channelopathies, disease of neuromuscular junction and peripheral nerve. In Seattle Children's Hospital 579 genes were included, which were revealed in Appendix 1 [13], and Gene lists for sequencing in Seoul National University were indicated in Appendix 2 (total 434 genes) [14]. Genomic DNA was extracted from peripheral blood leukocytes using a QIAamp® DNA Blood Midi Kit according to the manufacturer's instructions (Qiagen, Valencia, CA, USA). A DNA library was prepared for each sample by capturing the exons of the genes of interest using custom made DNA probes (Haloplex Agilent, Santa Clara, California, USA) in both centers. Samples were sequenced at 5–8 samples per lane on a GAIIx instrument (Illumina, San Diego,

California, USA) using 2×100 paired-ends reads in Seattle Children's Hospital and on the Hiseq 2000 sequencing system (Illumina HiSeq 2500, San Diego, California, USA) using 2 × 101 paired-end reads. Reads were aligned using Burrows-Wheeler Aligner (BWA, V.0.7.3) and data were analyzed with the Genome Analysis Toolkit (GATK, V.2.4.9) (Broad Institute, Cambridge, Massachusetts, USA) [15].

3. Variant annotation and identification of mutations

Variants were annotated using SeattleSeq [16] and wAnnovar [17]. Variants within the targeted region were further evaluated by cross-referencing to Single Nucleotide Polymorphism Database (dbSNP), the exome variant server, and the 1000 Genomes browser. PolyPhen-2 and SIFT (sorting intolerant from tolerant) scores were used for reference. We used the minor allele frequency (MAF) cut-off 0.2% for variants in genes with autosomal dominant or X-linked inheritance, and 0.5% for autosomal recessive variants [15]. If the variant exceed these frequencies, we considered the variant as non-pathogenic. Also, all variants were searched in the Human Gene Mutation Database (HGMD) [18, 19]. The sequence variants were interpreted based

on the guideline from American College of Medical Genetics [20]

4. Direct Sanger sequencing

For confirmation of variants, direct sequencing of the coding exon which included pathogenic variants of COL6A1, COL6A2, or COL6A3 was performed using primer pairs designed by the authors, which are available upon request. Polymerase chain reaction amplification was performed in a thermal cycler (Model 9700; Applied Biosystems, Foster City, CA, USA) and cycle sequencing was performed on an ABI Prism 3730xl DNA Analyzer using the BigDye Terminator Sequencing Ready Reaction Kit (Applied Biosystems). Sequencing for trio (the patient and parents) was available in 17 patients (patient No. 1-13, 15-18). Validation for the patient No. 14 was performed only in the patient and mother.

RESULT

1. Clinical features

Among 23 patients enrolled in the study, 14 (60%) were male and 9 (40%) were female. The mean age at last assessment was 12 years old (range, 2-29 years old) and mean follow-up duration was 7.7 years (range, 0-21 years). Clinical manifestations and phenotype of all patients are shown in Table 1. Fifteen patients (65%) had some perinatal abnormalities. Congenital hip dislocation, torticollis and arthrogryposis multiplex congenita were noted in 7 (30%), 5 (21%) and 3 (13%) patients, respectively. Perinatal distress such as poor sucking, respiratory difficulty or decreased muscle tone were reported in 9 (39%) of patients. The mean age at first symptom recognition was 24 months of age (range, 0-156 months). The most of patients (19 patients, 82%) initially presented with hypotonia since their birth (6 patients, 26%) or motor developmental delay before 24-month-old (13 patients, 56%). One patient (patient No. 1) was believed to have muscular disorder due to severe hyperlaxity of both hands and feet since her birth. The patient No. 8 initially presented with tip toeing since 6 years of age and

the patient No. 12 have showed progressive weakness in lower extremities since 7 years old. The patient No. 14 was brought to hospital due to limping gait starting since his age of 13. Six patients (26%) could walk alone before 15 months of age, whereas 3 (13%) never walked. Among 20 patients who had walked alone once, seven (35%) lost their walking ability at mean age of 9 years old (range, 4-11 years). Last motor performance was varies according to each patients, from running to near bed ridden state. Hyperlaxity of distal joints and contracture of proximal joints were noted each in 11 (48%) of patients. Twelve patients (52%) had various degree of scoliosis and 3 were treated surgically. Six patients (26%) used non-invasive positive pressure ventilator during sleep, with the mean starting age of 11 years (range, 9-13). Patients were categorized by clinical phenotype, as UCMD in 11 (48%), BM in 4 (17%), LGMD in 3 (13%) and undetermined in 5 (21%). None of the patients had cardiac problems, and intelligence was grossly normal in all patients except the patient 15, who could say a word only at 4 years of age and delayed cognitive function with perinatal history of neonatal respiratory difficulty and stay in neonatal intensive care unit. Muscle biopsy was done in all the

patients, with various performed age (range from 6 months to 29 years old). The pathology findings indicated various degree of dystrophic change and endomyseal fibrosis. The degree of size disproportion or muscle fiber integrity seemed to be not associated with the age of tissue securement. Immunohistochemistry of collagen VI was obtained in 20 patients, all revealed sarcolemma-specific collagen VI deficiency (SSCD).

Table1. Clinical features of the patients

No	Age/Sex	Past history			Motor function			Orthopedic problems			NIV	Clinical phenotype	IHC
		Perinatal problem	Initial symptom	Onset age (months)	Walk alone (months)	Loss of ambulation (years)	Last motor function	Distal joint hyperlaxity	Proximal joint contracture	Scoliosis			
1	9/F	Congenital torticollis	Hyperlaxity of hands and feet	0	14	Not yet	Run	+	-	+	-	UCMD	SSCD
2	16/M	Poor sucking Respiratory difficulty	Hypotonia	0	18	4	Near bed ridden	+	+	+, surgery	+	UCMD	SSCD
3	14/M	Poor sucking	Hypotonia	3	24	11	Arm elevation to head Neck flexor weakness	+	+	+, surgery	+	UCMD	SSCD
4	14/M	Congenital torticollis, respirory difficulty	Hypotonia	0	18	10	Arm elevation to head	+	+	+	+	UCMD	SSCD
5	3/F	Congenital hip dislocation, torticollis, arthrogryposis multiplex	Hypotonia	0	17	Not yet	Walk alone	+	+	-	-	UCMD	SSCD
6	4/F	Decreased fetal movement	Hypotonia	0	Never	NA	Arm elevation to shoulder Walk alone	+	-	-	-	UCMD	SSCD
7	10/F	-	Motor delay	18	16	Not yet	Step upstair wit holding the bar	-	-	-	-	LGMD	SSCD
8	8/F	Congenital hip dislocation	Tip toeing	60	18	Not yet	Walk with waddling	-	+	+	-	LGMD	SSCD

9	15/M	-	Motor delay	18	13	Not yet	Walk, step up with waddling	-	+	-	-	BM	SSCD
10	10/F	Congenital torticollis	Motor delay	12	12	Not yet	Walk alone	+	-	+	-	BM	SSCD
11	9/M	Hypotonia	Motor delay	12	15	Not yet	Run, step up	-	+	-	-	BM	SSCD
12	20/F	-	Lower motor weakness	84	13	Not yet	Walk alone, step with holding the bar	-	-	+, surgery	+	Undetermined	NA
13	11/M	Congenital hip dislocation, torticollis	Motor delay	13	NA	Not yet	Walk alone, step with holding the bar	+	-	+	-	Undetermined	SSCD
14	27/M	-	Limping gait	156	NA	Not yet	Walk, step up, foot drop only	-	-	-	-	Undetermined	SSCD
15	10/M	Congenital hip dislocation, arthrogryposis multiplex congenita, poor suking	Motor delay	24	NEVER	NA	Walk with assist in the pool	+	-	+	-	UCMD	SSCD
16	7/M	Decreased fetal movement, congenital hip dislocation	Motor delay	18	NEVER	NA	Walk alone Cannot run or step up	+	+	-	-	UCMD	SSCD
17	5/M	-	Motor delay	15	Before 15	Not yet	Walk alone Neck flexor weakness	-	-	-	-	LGMD	SSCD
18	8/F	-	Motor delay	24	17	6	Arm elevation to shoulder	-	+	-	-	BM	SSCD

19	12/M	-	Motor delay	8	13	11	Walk alone, step up with holding the bar	+	-	-	-	UCMD	NA
20	11/M	-	Motor delay	15	16	8	Arm elevation to shoulder	+	+	+, surgery	+	UCMD	SSCD
21	3/M	Congenital hip dislocation	Hypotonia	0	19	Not yet	Walk alone, partial Gowers sign	+	+	-	-	UCMD	NA
22	13/M	Hypotonia	Motor delay	6	24	9	Arm elevation to shoulder	-	-	+	+	Undetermined	SSCD
23	29/F	Congenital hip dislocation	Motor delay	18	48	Not yet	Walk alone few steps Near WB	-	-	+	-	Undetermined	SSCD

NIV = non-invasive ventilator, IHC = immunohistochemistry, UCMD= Ullrich congenital muscular dystrophy, SSCD = sarcolemma-specific collagen deficiency, BM = Bethlem myopathy, LGMD = limb girdle muscular dystrophy, NA = not available, WB = wheelchair bounded

2. Mutation profiles

Target gene panel sequencing was performed in all patients and 16 different variants (10 in *COL6A1*, 2 in *COL6A2*, 4 in *COL6A3*) were found. All except one variants were missense (11 patients, 48%) or splicing site mutation (11 patients, 48%). Genetic and immunohistochemistry findings of all patients are shown in Table 2. Fifteen patient (65%) had a COL6A1 variant, each 2 (13%) and 5 patients (22%) had *COL6A2* and *COL6A3* variant. The mutations of c.850G>A of COL6A1 was the most frequent single variant. All patients indicated dominant heterozygous variants. The patient No. 14 initially showed compound heterozygous mutations of c.350C>A and c.349T>A which were both novel variants, yet c.349T>A was seemed to be not damaging according to our variant identification protocol. The most of mutations (14 of 16 variants, 88%) were located in triple helical domain. Seventeen patients underwent direct Sanger sequencing with parents analysis. Among them, 13 patients had *De novo* mutations, whereas 4 patients inherited from one of their parents. Among 4 parents with the same variant, 2 showed similar symptoms with their children, while other 2 had no clinical symptoms (the patient No. 1 and 15). Five of total 16 variants

have never been reported so far with our knowledge, and other 5 variants of 11 previously reported mutations were first identified in our cases but they have already reported in the previous article of our group [13]. Muscle biopsy was done in all the patients, with various performed age (range from 6 months to 29 years old). The pathology findings indicated various degree of dystrophic change with endomyseal fibrosis. Immunohistochemistry of collagen VI was obtained in 20 patients, all revealed sarcolemma-specific collagen VI deficiency (SSCD).

Table 2. Mutation profiles of the patients

Patient No	Gene	Nucleotide change	Amino acid change	Affected domain	Inheritence	Reference
1	COL6A1	c.1461+3G>C	Splicing site	THD	Parternal, asymptomatic	CS151682*
2	COL6A1	c.958-2A>G	Splicing site	THD	De novo	Novel
3	COL6A1	c.958-2A>G	Splicing site	THD	De novo	Novel
4	COL6A1	c.850G>A	Missense, p.Gly284Arg	THD	De novo	CM050214
5	COL6A1	c.850G>A	Missense, p.Gly284Arg	THD	De novo	CM050214
6	COL6A1	c.850G>A	Missense, p.Gly284Arg	THD	De novo	CM050214
7	COL6A1	c.877G>A	Missense, p.Gly293Arg	THD	De novo	CM120696
8	COL6A1	c.868G>A	Missense, p.Gly290Arg	THD	De novo	CM050215
9	COL6A1	c.1002+1delG	Frameshift	THD	De novo	CD151671*
10	COL6A1	c.1003-2A>G	Splicing site	THD	Maternal, symptomatic	CS151669
11	COL6A1	c.1056+1delG	Splicing site	THD	Paternal, symptomatic	CD151670
12	COL6A1	c.850G>A	Missense, p.Gly284Arg	THD	De novo	CM050214

13	COL6A1	c.850G>A	Missense, p.Gly284Arg	THD	De novo	CM050214
14	COL6A2	c.350C>A	Missense, p.Ser117Try	N1	NA†	Novel
15	COL6A3	c.9329-4A>T	Splicing site	C4	Partenal, asymptomatic	CS151683*
16	COL6A3	c.6210+1G>A	Splicing site	THD	De novo	CS050010*
17	COL6A3	c.6188A>G	Missense, p.Try2063Cys	THD	De novo	Novel
18	COL6A3	c.6282+1G>C	Splicing site	THD	De novo	CS151672*
19	COL6A1	c.814G>A	Missense, p.Gly272Ser	THD	NA	Novel
20	COL6A1	c.859-2A>G	Splicing site	THD	NA	Novel
21	COL6A3	c.6210+1G>A	Splicing site	THD	NA	CS050010
22	COL6A1	c.868G>A	Missense, p.Gly290Arg	THD	NA	CS050215
23	COL6A2	c.801+1G>A	Splicing site	THD	NA	Novel

* first reported in previous study of our cohort, the same patient [13]
†father sample unavailable
COL6 = collagen VI, THD = triple helical domain, NA = not available

3. Phenotypic analysis with genetic data

Core clinical and genetic data for each phenotype is summarized in Table 3. UMCD patient showed first symptom at younger age (mean age 6 months) with more perinatal distress and congenital orthopedic problems than non-UCMD patients (mean age 40 months). Congenital orthopedic problems in 4 non-UCMD patients were torticollis or congenital dislocation of hip, and only one patient had both of them. The arthrogryposis congenita was noted only in UCMD patients. All 3 patients with arthrogryposis congenita also had another orthopedic problems (torticollis or congenital hip dislocation) and 2 of them never walked and the other patient could walk independently at last assessed age of 2.7 years. Eight UCMD patients (73%) never walked or loss of their walking ability at mean age of 9.4 years (range, 4.3-11.8) in comparison with 10 patients (83%) still could walk at last assessed age of 14 years in non-UCMD group. Mutation in *COL6A1* accounted for 8 (73%) in UCMD and 8 (66%) in non-UCMD patients. All two variants in *COL6A2* were found in undetermined patients only. Each 3 (27%) in UCMD and 2 (17%) in non-UCMD patients had variants in *COL6A3*. Most of affected domain (91%) was triple helical domain. One patient with UCMD

indicated variant in C4 domain, and one with non-UCMD had mutation in N1 domain. We also identified the mutation type according to phenotypes. Single amino acid substitution in exon accounted for 36% and 58% of UCMD and non-UCMD patients. Mutation in splicing site was documented in 7 (64%) of UCMD patients and 4 (33%) of non-UCMD patients. Three patients who never walked had an amino acid substitution in COL6A1 (patient No. 6), and two splicing site mutation in COL6A3 (patient No. 15 and 16). One frameshift mutation in COL6A1 was found in patient with BM. Other 3 patients with BM showed splicing site mutation.

Table 3. The summary of clinical and genetic data in each phenotype

Phenotype	Clinical data					Genetic data				
	Sex (M:F)	Mean onset age (months)	Congenital orthopedic problems (n, (%))	Other perinatal history (n, (%))	Last motor performance (n, (%, mean age of last exam))	COL6A1 (n, (%)	COL6A2 (n, (%)	COL6A3 (n, (%)	Affected domain (n, (%))	Mutation type (n, (%))
UCMD (n=11)	8:3	6	7 (64)	7 (64)	NW, 3 (27, 7 years) LOA, 5 (46, 13 years) SW, 3 (27, 5 years)	8 (73)	0 (0)	3 (27)	THD, 10 (91) C4, 1 (9)	AAS, 4 (36) Splicing, 7 (64)
Non-UCMD (n=12)	6:6	40	4 (33)	2 (17)	LOA, 2 (17, 10 years) SW, 10 (83, 14 years)	8 (66)	2 (17)	2 (17)	THD, 11 (92) N1, 1(8)	AAS, 7 (58) Splicing, 4 (33) FS, 1 (9)
BM (n=4)	2:2	16	1 (25)	1 (25)	LOA, 1 (25, 7 years) SW, 3 (75, 12 years)	3 (75)	0 (0)	1 (25)	THD, 4 (100)	Splicing 3 (75) FS, 1 (25)
LGMD (n=3)	2:2	74	1 (25)	0 (0)	SW 3 (100, 7 years)	22 (67)	0 (0)	1 (33)	THD, 3 (100)	AAS, 3 (100)
UD (n=5)	2:2	30	2 (50)	1 (25)	LOA 1 (20, 13 years) SW, 4 (80, 22 years)	3 (60)	2 (40)	0 (0)	THD, 4 (80) N1, 1(20)	AAS, 4 (80) Splicing, 1 (20)

UCMD = Ullrich congenital muscular dystrophy, BM = Bethlem myopathy, LGMD = limb girdle muscular dystrophy, UD = undetermined, NW = never walked, LOA = loss of ambulation, SW = still walking, THD = triple helical domain, AAS = amino acid substitution, FS = frameshift

DISCUSSION

Despite the true incidence of total CMDs and COL6 myopathy has not been established and might vary across the countries, there is no doubt that COL6 myopathy is one of the most common type CMDs. It has been considered as second most common type in Japan [21], accounting for 9.4% of all CMDs, and UCMD with mutation in collagen VI genes accounts for 16% of all CMD cohort in Italy [22]. According to the previous study, COL6 myopathy is the most common type in our cohort [13]. Considering its frequency, profound understanding and investigation for COL6 myopathy is crucial for patients' care as well as CMD research.

The concept of COL6 myopathy has evolved over years. Each UCMD and BM had described separately at first [2, 4], yet both have been considered to be in a line of clinical spectrum since the causative genes found [9, 11]. In genetic backgrounds, it is well known that UCMD and BM might be caused by dominant and recessive trait either, unlike their first report of recessive inheritance pattern in UCMD and dominant inheritance in BM [8-11]. Phenotypes of COL6 myopathies also have been expanded

over years, referred to intermediate collagen VI-related myopathy, mild UCMD or severe BM [23]. Moreover, phenotypes such as LGMD-like or myosclerosis myopathy with mutation in collagen VI gene were reported so that simple clinical approach could miss the diagnosis. In our cohort, five patients were categorized as undetermined. Among them, the patient No. 13 presented limping gait since age of 13 years without any perinatal problems, and he is still able to walk or step up at present (27-year-old), but showed distal lower leg weakness and foot drops without proximal weakness, mimicking distal myopathy. The patient No. 23 was brought the hospital due to moderate to severe motor developmental delay at age of 18 months. She could walk alone past over 4 years of age, but still walk independently without respiratory compromise. On the other hands, the patient No.12 presented with progressive weakness of lower extremities since her age of 7 years. Despite of progressive weakness, she can still walk independently and step up with holding the bar, at age of 20 years. However her respiratory function have rapidly declined as functional vital capacity below 25%, so she have used night time non-invasive positive pressure ventilator since her age of 13 years. Likewise,

COL6 myopathy showed diverse phenotypes and severities without typical characteristics of collagenopathy including joint problems and clinical course of skeletal muscle weakness following respiratory compromise, as well as classical UCMD, BM, or intermediate phenotype. We expand the clinical spectrum with those cases of COL6 myopathies, helpful in clinic to diagnosis and management, as well as broadening academic perspectives.

According to expansion of genetic study especially gene panel or whole exome sequencing, heterozygous mutations have been frequently found [24], and recent study indicated that dominant mutation is the major genetic backgrounds even in UCMD [25, 26]. In our study, all patients revealed heterozygous mutation, supporting evidence of majority of dominant variant in UCMD. It has been well known that glycine substitutions in the triple helical domains (THD) are most common site for mutations of COL6 myopathy [12, 27], consistent with the present study. The variants distribution for each gene varied by studies each, yet variants in *COL6A2* accounted for 13% in our study, less frequent compared with 35-48% in other studies [12, 21, 28]. The patient No. 1 and 15 had heterozygous variant

(c.1461+3G>C in COL6A1 and c.9329-4A>T in COL6A3) inherited from their parents without any clinical symptoms. We conclude that these variants pathogenic based on previous report, patients' immunohistochemistry (IHC) findings and our variant analyzing protocol. Therefore the condition was thought to "incomplete penetrance". Incomplete penetrance in collagenopathy was only reported in COL6A1 splicing site mutation leading to premature stop codon [29]. In three UCMD family with homozygous mutation of COL6A3, all parents who were heterozygous carrier showed no clinical symptom yet the one young offspring with heterozygous variants have started to indicated joint symptoms [30]. Our 2 splicing site mutation in COL6A1 and COL6A3 might be another evidence of variable penetrance. Further study for unknown modifier of COL6 myopathy is needed.

In pathology, similar to the inheritance type, SSCD have been rapidly increased since its first description [31]. It is well known that complete deficiency (CD) and SSCD are associated with recessive and dominant mutation each, although it doesn't always coincide. Our patients, all with heterozygous mutations, indicated SSCD in this context. The degree of SSCD was also diverse (Fig.

1), and some IHC findings were very tricky to diagnosis without genetic analysis, might be due to interstitial fibrosis in some patients. In these cases, pathology gave only limited information and genetic study could performed a key role for diagnosis. In fact, genetic technology such as gene panel sequencing or whole-exome sequencing have been ranked gold standard to diagnosis of CMDs. Nevertheless, pathologic findings can provide some clues for diagnosis with clinical information together, because target gene panel sequencing cannot confirm the final diagnosis by itself. Therefore, overall comprehension in patients' phenotype, biopsy findings including IHC, and appropriate genetic test and interpretation is needed to exact diagnosis.

Over 300 pathogenic variants in *COL6A1*, *COL6A2*, and *COL6A3* have been reported [32]. The correlation between genotype and phenotype have been difficult to analyze and seemed to be not so clear, due to their heterogeneity and genetic overlapping across the phenotypes. So far, glycine substitution in a critical region near the N-terminal THD has thought to be associated with severe phenotype in some patients might be due to reduced collagen VI as well as assembly

abnormality. But in the same study, another patients with same mutation showed phenotype with normal amounts of collagen [33]. Two patients with homozygous premature stop codon (PTC) mutations were reported as severe phenotypes, yet the other patient with homozygous PTC showed mild phenotypes [12]. Recent study in Japan revealed that there were no clear genotype-phenotype correlations [21]. No definite genotype-phenotype association was observed in our study, either. In our cohort, 9 patients (39%) revealed glycine substitution in THD domain. No patient had homozygous mutations and only one patient had heterozygous frameshift mutation. Most of them was located in THD and showed various phenotypes and clinical severities. Three patients who never walked in the present study had one missense glycine substitution and two splicing mutations each. Among them, the patient with common missense glycine substation (c.850G>A in *COL6A1*), who is 4-year-old girl, could elevate her arm up to shoulder only. The heterozygous missense mutation of c.850G>A in *COL6A1* is one of the most common variant in various phenotype [34]. We found the variant in 5 patients (22%, patient No. 4, 5, 6, 12, and 13), who showed extremely variable clinical features from never walking (patient

6) to walking freely at age of 20 (patient No. 12). Pathology findings are also extremely heterogeneous, in both Hematoxylin-Eosin (HE) stain and collagen VI IHC. The Fig. 2 indicated HE and IHC of collagen VI findings in 3 patients with c.850G>A heterozygous variants, which revealed various degree of dystrophic change and deficiency of collagen VI. The pathology of the patient 6 indicated severe dystrophic change with moderate to severe endomyseal fibrosis, whereas that of the patient 13 revealed relatively mild dystrophic change only and less obvious deficiency in collagen VI expression. The phenotypic heterogeneity in the same mutation is s also indicative of the presence of other modulator for COL6 myopathy.

To predict the disease severity and prognosis, phenotypic classification have been considered the priority. As previously shown in Table 3, UCMD patients seemed to have sever phenotype compared with non-UCMD patients. Nevertheless, it didn't always coincide. The patient No. 1 presented with UCMD could still walk and hop in one leg at her age of 9 years, whereas the patient No. 18 initially presented with mild phenotype followed by rapid deterioration of muscle power and wheelchair bounded at 6 years old. Also, patients showed variable clinical

courses in age of first walking, max motor performance and velocity of deterioration even in the same phenotype. Therefore, early phenotypic classification could not show patients' prognosis exactly and long term follow-up and serial assessment for patients might be only way to predict outcome and provide appropriate management. Exceptionally, all patients with arthrogryposis congenita had also combined other orthopedic problems and devastating clinical courses. We carefully suggest that multiple orthopedic problems especially multiple joints contracture might be helpful to predict poor outcome in early period of disease.

In conclusion, our data might expand the clinical spectrum with some novel variants, as well as reinforcement of previous study relating to heterogeneity and complexity of COL6 myopathy. Clinical diversity and incomplete penetrance indicated the presence of additive modifier in COL6 myopathy. There were no clear genotype-phenotype correlation and phenotypic classification was hard to predict patients' outcome. Exceptionally, multiple congenital orthopedic problems could be the predictor for poor outcome.

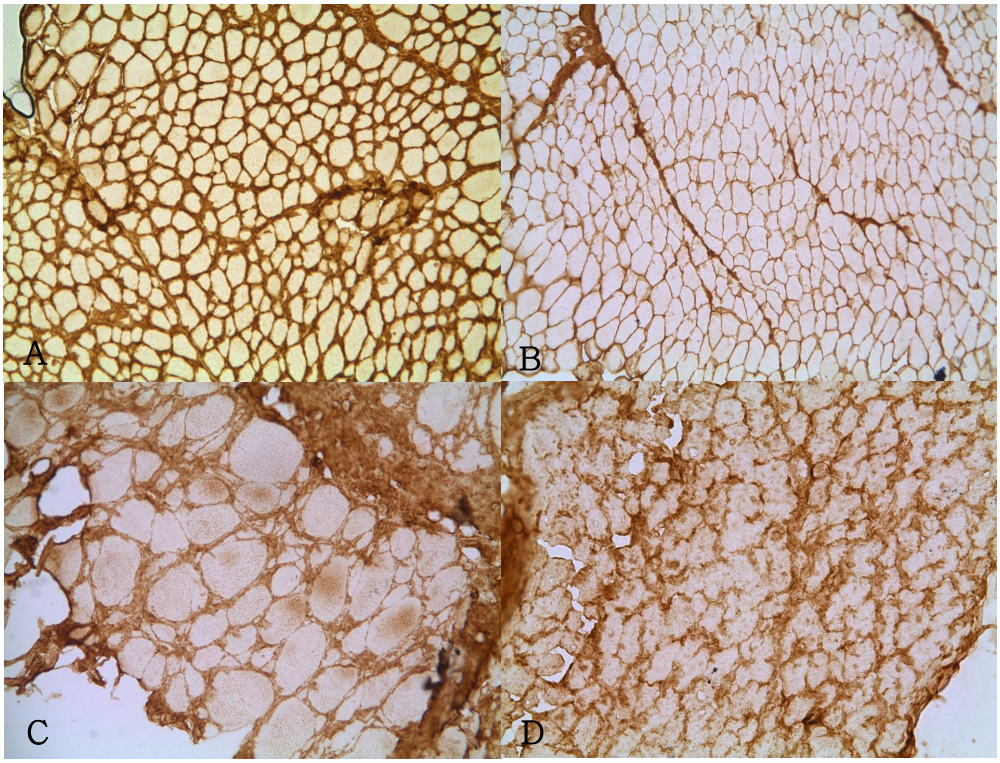

Figure 1. Heterogeneity of collagen VI immunohistochemistry (IHC) findings.

Each indicated (A) relatively preserved integrity of muscle fibers and not clear collagen VI deficiency (patient No. 4, muscle biopsy done at 10 years old) (B) relatively preserved integrity and more obvious collagen VI deficiency (patient No. 15, muscle biopsy done at 4 years old) (C) severe fiber size variation with fibrosis and sarcolemma-specific collagen VI deficiency (patient No. 17, muscle biopsy done at 2 years old) (D) more decreased collage VI (patient No. 20, muscle

biopsy done at 2 years old).

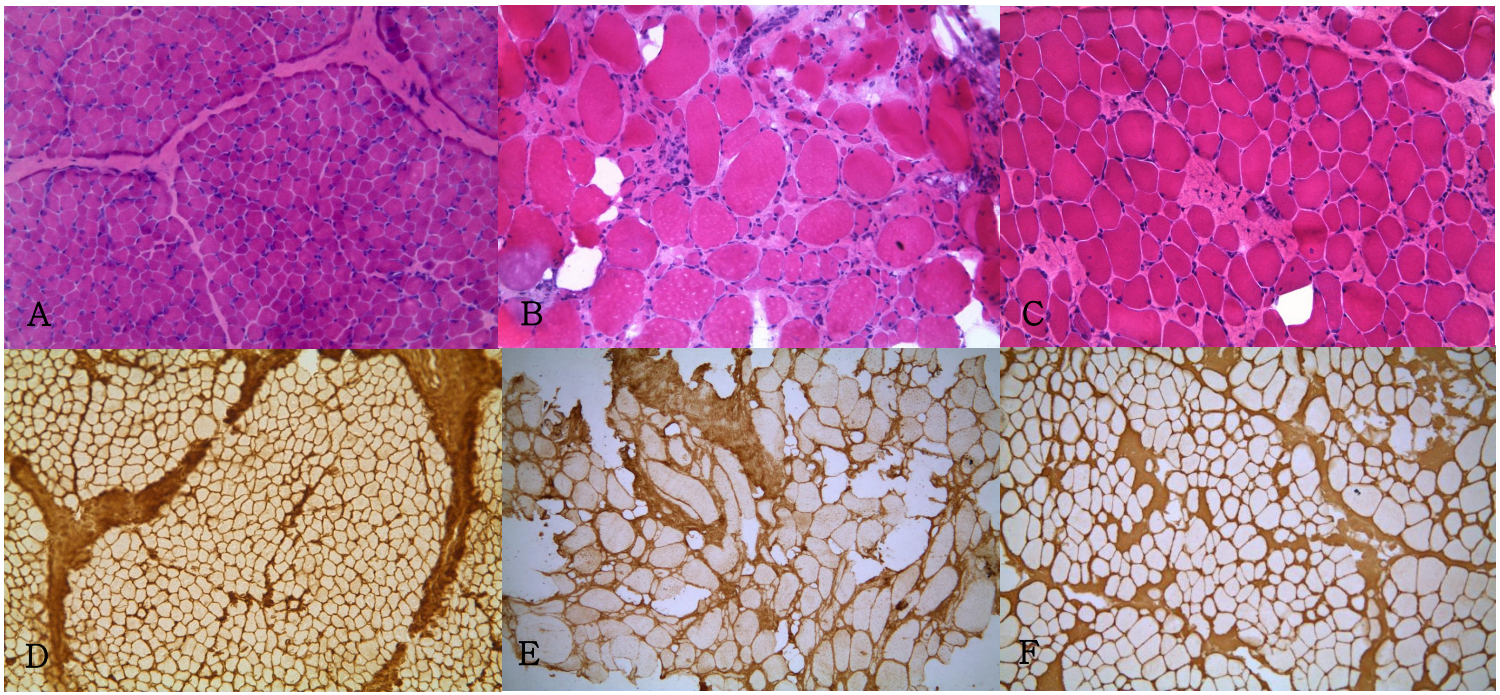

Figure 2. Pathology findings of the patients with c.850G>A in *COL6A1*.

Hematoxylin-Eosin stain and immunohistochemistry of collage VI findings in the patient 5 (A, D, muscle biopsy done at 7 months old), the patient 6 (B, E, muscle biopsy done at 2 years old), and the patient 13 (C,

F, muscle biopsy done at 5 years old), which showed different degree of dystrophic change and collagen VI deficiency each other.

Appendix 1. List of Genes for targeted gene panel sequencing in Seattle Children's Hospital (total 579 genes)

Congenital Myoapthy (30)

ACTA1, ACTG2, ATP2A1, BIN1, CCDC78, CFL2, CLCN1, CNTN1, DNM2, FAM123B, FLNC, GNE, OXD10, HRAS, KBTBD13, LDB3, MEGF10, MTM1, MTMR14, MYF6, MYH2, MYH7, NEB, TIA1, TNNT1, TPM2, TPM3, TTN, VCP, VMA21

Muscular dystrophy (48)

ANO5, BAG3, CAPN3, CAV3, CHKB, CNBP, COL6A1, COL6A2, COL6A3, CRYAB, DAG1, DES, DMD, DMPK, DNAJB6, DPM2, DPM3, DYSF, EMD, FHL1, FKBP14, FKRP, FKTN, GTDC2, ISPD, ITGA7, ITGA9, KLHL9, LAMA2, LARGE, LMNA, MTAP, MYOT, PABPN1, PLEC, PLOD3, POMGNT1, POMT1, POMT2, SECISBP2, SGCA, SGCB, SGCD, SGCG, SPP1, SYNE2, TCAP, TRIM32

Congenital myopathy or muscular dystrophy (2)

RYR1, SEPN1

Metabolic myopathy (202)

AARS2, ACACA, ACAD9, ACADM, ACADVL, ACSF3, ACY1, ADCK3, AGK, AGL, AIFM1, ALDH1B1, ALDOA, ALG1, ALG11, ALG12, ALG13, ALG2, ALG3, ALG6, ALG8, ALG9, ALPL, AMACR, AMPD1, ATP5A1, ATP5E, ATP5G3, ATPAF2, B4GALT1, BCS1L, BRP44L, C10orf2, C12orf65, CACNA1S, COA5, COG1, COG4, COG5, COG6, COG7, COG8, COQ2, COQ4, COQ6, COQ9, COX10, COX14, COX15, COX20, COX4I2, COX6B1, COX7B, CPT1A, CPT1B, CPT2, CYC1, CYCS, D2HGDH, DARS2, DGUOK, DMGDH, DNA2, DOLK, DPM1, EARS2, ENO3, ETFA, ETFB, ETFDH, FARS2, FASTKD2, FOXRED1, GAA, GALC, GBE1, GFER, GFM1, GPR172B, GYG1, GYS1, HADH, HADHA, HADHB, HARS2, IARS, ISCU, LAMP2, LARS2, LDHA, LDHB, LIAS, LPIN1, LRPPRC, LYRM4, LYRM7, MARS, MATR3, MGAT2, MGME1, MPV17, MRPL12, MRPL3, MRPL44, MRPS16, MRPS22, MTFMT, MTHFD1, MTHFR, MTO1, NDUFA1, NDUFA10, NDUFA11, NDUFA12, NDUFA2, NDUFA4, NDUFA9, NDUFAF1, NDUFAF2, NDUFAF3, NDUFAF4, NDUFAF5, NDUFAF6, NDUFB3, NDUFB9, NDUFS1, NDUFS2, NDUFS3, NDUFS4, NDUFS6, NDUFS7, NDUFS8, NDUFV1, NDUFV2, NEU1, NFU1, NUBPL, OAT, OPA1, OXCT1, PDSS1, PDSS2, PEX12, PEX14, PEX26, PEX3, PEX5, PEX6, PEX7, PFKM, PGAM2, PGK1, PGM1, PHKA1, PHKA2, PHKB, PHKG2, PHYH, PMM2, PNPLA2, PNPT1, POLG, POLG2, PSAP, PTEN, PTRF, PUS1, PYGL, PYGM, RMND1, RRM2B, RSPH9, SCO1, SCO2, SDHA, SDHAF1, SDHAF2, SDHB, SDHC, SDHD, SLC22A5, SLC25A20, SLC25A3, SLC25A4, SUCLA2, SUCLG1, SURF1, TACO1, TARS2, TAZ, TIMM44, TK2, TMEM165, TMEM70, TSFM, TTC19, TYMP, UQCRB, UQCRC2, UQCRQ, VARS2,

YARS2

Motor neuron or Peripheral nerve disease (75)

AARS, AFG3L2, APTX, ARHGEF10, ATL1, ATXN2, BSCL2, CCT5, COLQ, DCTN1, DHTKD1, DNAJB2, DNMT1, DYNC1H1, EGR2, ELAVL4, ERLIN2, FAM134B, FGD4, FOXE1, GAN, GARS, GDAP1, GJB1, HARS, HINT1, HOXB1, HSPB1, HSPB3, HSPB8, HSPG2, IGHMBP2, IKBKAP, INF2, KARS, KIF1B, KIF5A, LITAF, MED25, MFN2, MPZ, MTMR2, MYH14, NAGA, NDRG1, NEFL, NGF, NIPA1, PDK3 , PLEKHG5, PLP1, PMP22, PNPLA6, PRX, RAB7A, REEP1, SACS, SBF2, SH3TC2, SLC12A6, SLC5A7, SMN1, SMN2, SNX25, SPTLC1, SPTLC2, SYNE1, TDP1, TFG, TGM6, TRPV4, UBA1, VRK1, WNK1, YARS

Myasthenic syndrome (12)

AGRN, CHAT, CHRNA1, CHRNB1, CHRND, CHRNE, DOK7, DPAGT1, GFPT1, LAMB2, MUSK, SCN4A

Cardiomyopathy (3)

MYBPC3, MYLK2, RYR2

Other candidate gene (207)

ACAD10, ACAD11, AIFM2, ATP5B, ATP5C1, ATP5D, ATP5F1, ATP5G1, ATP5G2, ATP5H, ATP5I, ATP5J, ATP5J2, ATP5L, ATP5O, ATP5S, ATPAF1, ATPIF1, C14orf2, CARS, CARS2, CD36, CHCHD1, CHCHD7, CMC1, CMC2, COA1, COA3, COA6, COQ10A, COQ10B, COQ3, COQ5, COQ7, COX11, COX16, COX17, COX18, COX19, COX4I1, COX5A, COX5B, COX6A1, COX6A2, COX6B2, COX6C, COX7A1, COX7A2, COX7A2L, COX7B2, COX7C, COX8A, COX8C, ECi1, ECSIT, EPRS, FARSA, FARSB, FRG1, FUNDC2, HINT3, IARS2, ICT1, LACTB, LYRM1, MCAT, MDH2, METTL17, MNF1, MRP63, MRPL1, MRPL10, MRPL11, MRPL13, MRPL14, MRPL15, MRPL16, MRPL17, MRPL18, MRPL19, MRPL2, MRPL20, MRPL21, MRPL22, MRPL23, MRPL24, MRPL27, MRPL28, MRPL30, MRPL32, MRPL33, MRPL34, MRPL35, MRPL36, MRPL37, MRPL38, MRPL39, MRPL4, MRPL40, MRPL41, MRPL42, MRPL43, MRPL45, MRPL46, MRPL47, MRPL48, MRPL49, MRPL50, MRPL51, MRPL52, MRPL53, MRPL54, MRPL55, MRPL9, MRPS10, MRPS11, MRPS12, MRPS14, MRPS15, MRPS17, MRPS18A, MRPS18B, MRPS18C, MRPS2, MRPS21, MRPS23, MRPS24, MRPS25, MRPS26, MRPS27, MRPS28, MRPS30, MRPS31, MRPS33, MRPS34, MRPS35, MRPS36, MRPS5, MRPS6, MRPS7, MRPS9, MRS2, MTCH1, MTERF, MTG1, MTHFD1L, MTHFS, MTIF3, MTRF1L, NARS, NARS2, NDUFA3, NDUFA4L2, NDUFA5, NDUFA6, NDUFA7, NDUFA8, NDUFAB1, NDUFB1, NDUFB10, NDUFB11, NDUFB2, NDUFB4, NDUFB5, NDUFB6, NDUFB7, NDUFB8, NDUFC1, NDUFC2, NDUFS5, NDUFV3, NIPSNAP3A, NRF1, OXA1L, OXSM, PARS2, PGAM1, POLRMT, PRELID1, PRELID2, PTCD1, PTCD2, PTCD3, PTRH2, QARS, RARS, SLIRP, SMCR7, SMCR7L,

SUCLG2, TARS, TARSL2, TIMM21, TIMM23, TOMM70A, TOP1MT, TYMS, UQCR10, UQCR11, UQCRC1, UQCRFS1, UQCRH, USMG5, VARS, WARS, WARS2, YME1L1

Appendix 2. List of Genes for targeted gene panel sequencing in Seoul National University Children's Hospital (total 434 genes)

Muscular dystrophies (40) *DMD, EMD, FHL1, LMNA, SYNE1, SYNE2, TMEM43, TOR1A1P1, DUX4, SMCHD1, PTFR, MYOT, CAV3, DNAJB6, DES, TNPO3, HNRPDL, CAPN3, DYSF, SGCG, SGCA, SGCB, SGCD, TCAP, TRIM32, FKRP, TTN, POMT1, ANO5, FKTN, POMT2, POMGNTI, PLEC, TRAPPCI1, GMPPB, DAGI, DPM3, ISPD, VCP, LIMS2, GAA*
Congenital muscular dystrophies (28) *LAMA2, COL6A1, COL6A2, COL6A3, SEPN1, FHL1, ITGA7, DNM2, TCAP, LMNA, FKTN, POMT1, POMT2, FKRP, POMGNTI, ISPD, POMGNT2, B3GNT1, GMPPB, LARGE, DPM1, DPM2, ALGI3, B3GALNT2, TMEM5, POMK, CHKB, ACTA1, TRAPC11*
Distal myopathies (14) *DYSF, TTN, GNE, MYH7, MATR3, T1A1, MYOT, NEB, CAV3, LDB3, ANO5, KLHL9, DNM2, FLNC, VCP*
Other myopathies (21) *ISCU, MSTN, FHL1, BAG3, ACVR1, MYOT, FLNC, LDB3, LAMP2, VCP, CAV3, SEPN1, DES, VMA21, PLEC, PABPN1, TTN, RYR1, CLN3, TRIM54, TRIM63,*
Myotonic syndromes (5) *DMPK, ZNF9, CAV3, HSPG2, ATP2A1*
Ion channel muscle disease (7) *CLCN1, SCN4A, CACNA1S, CACNA1A, KCNE3, KCNA1, KCNJ18*
Malignant hyperthermia (2) *RYR1, CACNA1S*
Metabolic myopathies (26) *GAA, AGL, GBE1, PYGM, PFKM, PHKA1, PGM1, GYG1, GYS1, PRFAG2, RBCK1, PGK1, PGAM2, LDHA, ENP3, CPT2, SLC22A5, SLC25A20, ETFA, ETFB, ETFH, ACADVL, ABHD5, PNPLA2, ETFDH, LPIN1,*
Hereditary cardiomyopathies (69) *MYH6, MYH7, TNNT2, TPM1, MYBPC3, PRKAG2, TTNI3, MYL3, TTN, MYL2, ACCTC1, CSRP3, TNNC1, MYH6, VCL, MYLK2, MYOZ2, JPH2, PLN, NEXN, ANKRD1, ACTN2, NDUFAF1, TSFM, AARS2, MRP13, COX15, MTO1, MRPL44, LMNA, ACTN2, LDB3, TNNT2, SCN5A, DES, EYA4, SGCD, CSRP3, TCAP, ABCC9, PLN, ACTC1, TMPO, PSEN2, VCL,*

TPM1, TNNC1, CRYAB, MYBC3, FCMD, TAZ, LAMA4, ILK, MYPN, RBM0, ANKRD1, TNN!3, MYH6, NEXN, SYNE1, MURC, DOLK, GATAD1, SDHA, TTN13, GAA, DTN4, FLNA

Congenital myasthenic syndromes (19)

CHRNA1, CHRNB1, CHRND, CHRNE, RAPSN, CHAT, COLQ, MUSK, DOK7, AGRN, DPAGT1, LAMB2, SCN4A, CHRNG, PLEC, ALG2, ALG14, SYT2, PREPL

Motor neuron disease (51)

SMN1, IGHMBP2, PLEKHG5, HSPB8, HSPB1, HSPB3, AARS, GARS, REEP1, SLC5A7, DCTN1, UBA1, ATP7A, DNAJB2, TRPV4, DYNC1H1, BICD2, FBXO38, ASAH1, VAPN, EXOSC8, SOD1, ALS2, SETX, FUS. ANG, TARDBP, FIG4, OPTN, ATXN2, VCP, UBQLN2, SIGMAR1, CHMP2B, PFN1, MATR3, NEFH. PRPH, C9orf72, CHCD10, SQSTM1, AT, GLE1, ERBB3, PIP5K1C, EXOSC3, SLC52AA3, SLC52A2, HEXB

Hereditary ataxia (45)

ATXN1, ATXN2, ATXN3, SPTBN2, CACNA1A, ATXN7, ATXN8OS, ATXN10, TTBK2, PPP2R2B, KCNC3, PRKCG, ITPR1, TBP, IFRD1, KCND3, PDYN, EEF2, FGF14, AFG3L2, BEAN1, TK2, ELOVL4, TGM6, NOP56, ELOVL5, CCDC88C, KCNA1, CACNB4, SLC1A3, FXN, TTPA, C10orf2, APTX, SETX, SYNE1, ADCK3, TDP1, SIL1, POLG. ATM, MRE11A, SACS, PHYH, PEX7, RNF216

Hereditary motor and sensory neurpathies (63)

PMP22, MPZ, LITAF, EGR2, NEFL, HOXD10, ARHGEF10, FBLN5, DNM2, YARS, INF2, GNB4, DGDAP1, MTMR2, SBF2, SBF1, SH3TC2, NDRG1, PRX, HK1, FGD4, FIG4, SURF1, GJB1, AIFM1, PRPS1, PDK3, KIF1B, MFN2, RAB7A, TRPV4, GARS, HSPB1, HSPB8, AARS, DYNC1H1, LRSAM1, DHTKD1, TRIM2, TFG, MRAS, KIF5A, LMNA, MED25, DNAJB2, HINT1, KARS, PLEKHG5, COX6A1, IGHMBP2, SPTLC1, SPTLC2, ATL1, KIF1A, WNK1, IKBKAP, NGF, DNMT1, SLC12A6, GJB3, GAN, CTDP1, VRK1, SEPT9

Hereditary paraplegias (45)

ATL1, SPAST, NIPA1, KIAA0196, KIF5A, RTN2, HSPD1, BSCL2, REEP1, ZFYVE27, SLC33A1, CYP7B1, SPG7, SPG11, SFYVE26, ERLIN2, SPG20, SPG21, B4GALNT1, DDHD1, KIF1A, PNPLA6, C19orf12, GJC2, NT5C2, GBA2, AP4B1, AP5Z1, TECPR2, AP4M1, AP4E1, AP4S1, DDHD2, C12orf65, CYP2U1, ARL6IP1, AMPD2, ENTPD1, ALD3A2, L1CAM, PLP1, MYPAP, AFG3L2, SACS

Other neuromuscular disorders (26)
TOR1A, SGCE, IKBKAP, TTR, KIF21A, PHOX2A, TUBB3, TPM2, MYH3, TNNI2, TNNT3, SYNE1, MYH8, POLG, SLC25A4, C10orf2, POLG2, TK2, SUCLA2, OPA1, STIM1, ORAI1, PUS1, CHCHD10, CASQ1, YARS2

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초 록

서론: Collagen VI 연관형 근병증은 선천성 근이영양증 가운데 흔한 원인을 차지하고 있으며, 임상상이 다양하며 뚜렷한 치료방법이 없어 치료 반응을 확인할 수 없으므로 그 진단이 매우 어려운 경우가 많다. 본 연구에서는, 유전자 검사로 확진된 collagen VI 연관형 근병증 환자들의 임상적 특징 및 유전형의 다양성에 대해 기술 및 분석하고자 하였다.

방법: 2000년 8월 1일부터 2016년 7월 31일까지 서울대학교 어린이병원에 방문한 환자 중 선천성 근이영양증으로 진단받았으나 세부 아형이 확진되지 않은 환자를 대상으로 유전자 패널 염기분석을 시행하였으며, 이 중 collagen VI 연관형 근병증의 원인유전자로 알려진 *COL6A1, COL6A2, COL6A3*의 돌연변이가 확진된 23명의 환자를 연구에 포함하였다. 의무기록 및 유전형에 대한 정보를 후향적으로 수집하여 분석하였다.

결과: 23명 중 19명의 환자는 출생시부터의 근긴장 저하 (6명, 26%) 혹은 24개월 이전에 인지된 운동발달지연 (13명, 56%)를 주소로 병원에 내원하였다. 10명(34%)은 출생 시 하나 이상의 선천적 골격계 질환(선천 사경, 선천성 고관절 탈구, 관절 구축)에 대한 병력이 있었다. 세 명(13%)의 환자는 혼자 걷는 능력을 획득하지 못하였으며, 혼자 걸을 수 있었던 환자 중 7명(35%)은 평균 10세 경 걷는 능력

을 상실하였다. 원위부 관절의 과신전, 근위부 관절의 구축, 척추측만증이 각각 11명(48%)의 환자에서 확인되었으며, 척추측만증이 있던 환자 중 3명은 수술적 치료를 시행받았다. 여섯 명(26%)의 환자는 수면 시 기계보조환기를 사용하였다. 환자 개개인에 따라 다양한 임상상 및 경과를 보였다. 근육조직검사상 모든 환자는 근섬유막 특이 collagen 결핍을 보였다. 임상적으로, 11명의 환자 (48%)가 Ullirch 타입 근이영양증으로 분류되었고 4명 (17%)은 Bethlem 타입 근병증, 3명 (13%)은 지대근이영양증, 나머지 5명 (22%)는 어디에도 속하지 않는 것으로 분류되었다. 15명(65%)의 환자가 *COL6A1*에 대한 돌연변이를 가지고 있었으며, *COL6A2, COL6A3*에 대한 돌연변이는 각각 3명(13%), 5명(22%)에서 보였다. 모든 환자의 돌연변이는 이형 돌연변이였으며 대부분의 돌연변이는 3중 나선 구조 도메인에 위치했다. 이번 연구에서 총 5개의 돌연변이는 이전에 보고된 적이 없는 것이었다. 돌연변이의 유전형과 임상형 간 의미 있는 관련성은 발견되지 않았으며 출생 시 선척적 다중 관절 구축이 있었던 경우 이른 시기부터 심한 증상을 보이는 경우가 많았다.

결론: 이번 연구를 통해 collagen VI 연관형 근병증의 임상적, 조직학적, 그리고 유전학적 다양성을 확인하였다. 유전형과 임상상의 연관성은 뚜렷하지 않았으나 출생 시 다양한 근골격계 문제, 특히 다

수의 관절 구축이 있는 경우 이후의 좋지 않은 예후를 보이는 경우가 있어 비교적 이른 시기에 예후 예측 인자로 활용할 수 있을 것으로 기대된다.

주요어: collagen VI-연관형 근병증, Ullich 근이영양증, Bethlem 근병증, *COL6A1, COL6A2, COL6A3*